"EFFECT OF CALCAREA CARBONICA ON OSTEOPOROTIC ACTIVITIES IN OVARIECTOMISED ALBINO RAT MODEL OF OSTEOPOROSIS IN 1X, 30 AND 0/1 POTENCIES"

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENT

FOR THE AWARD OF THE DEGREE OF

DOCTOR OF MEDICINE (HOMOEOPATHY) IN

MATERIA MEDICA

By

Dr. ALPHY MATHEW

UNDER THE GUIDANCE OF

Dr. WINSTON VARGHEESE, M.D., (Hom.)

PROFESSOR

DEPARTMENT OF MATERIA MEDICA

SARADA KRISHNA HOMOEOPATHIC MEDICAL COLLEGE,
KULASEKHARAM, TAMIL NADU

SUBMITTED TO

THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY, CHENNAI

2019

ENDORSEMENT BY THE HEAD OF THE DEPARTMENT AND THE INSTITUTION

This is to certify that the Dissertation entitled **"EFFECT OF CALCAREA CARBONICA ON OSTEOPOROTIC ACTIVITIES IN OVARIECTOMISED ALBINO RAT MODEL OF OSTEOPOROSIS IN 1X, 30 AND 0/1 POTENCIES"** is a bonafide work carried out by **Dr. ALPHY MATHEW,** student of **M.D (Hom.)** in **MATERIA MEDICA (2016 – 2019)** in Sarada Krishna Homoeopathic Medical College, Kulasekharam, Tamil Nadu under the supervision and guidance of **Dr. WINSTON VARGHEESE, M.D., (Hom.), PROFESSOR, DEPARTMENT OF MATERIA MEDICA** in partial fulfillment of the Regulations for the award of the Degree of **DOCTOR OF MEDICINE (HOMOEOPATHY)** in **MATERIA MEDICA**. This work confirms to the standards prescribed by **THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY, Chennai.**

This has not been submitted in full or part for the award of any degree or diploma from any University.

Dr. C. R.KRISHNA KUMARI AMMA, M.D. (Hom.)
Professor & HOD Department of Materia Medica

Dr. N.V. SUGATHAN, M.D. (Hom.)
Principal

Place: Kulasekharam
Date:

CERTIFICATE BY THE GUIDE

This is to certify that the Dissertation entitled **"EFFECT OF CALCAREA CARBONICA ON OSTEOPOROTIC ACTIVITIES IN OVARIECTOMISED ALBINO RAT MODEL OF OSTEOPOROSIS IN 1X, 30 AND 0/1 POTENCIES"** is a bonafied work of **Dr. ALPHY MATHEW.** All her work has been carried out under my direct supervision and guidance. Her approach to the subject has been sincere, scientific and analytic. This work is recommended for the award of degree of **DOCTOR OF MEDICINE (HOMOEOPATHY)** in **MATERIA MEDICA** of **THE TAMILNADU DR. M.G. R MEDICAL UNIVERSITY, CHENNAI**.

Place: Kulasekharam
Date:

Dr. WINSTON VARGHEESE., M.D. (Hom.)
Professor, Department of Materia Medica

DECLARATION

I, **Dr. ALPHY MATHEW** do hereby declare that this Dissertation entitled **"EFFECT OF CALCAREA CARBONICA ON OSTEOPOROTIC ACTIVITIES IN OVARIECTOMISED ALBINO RAT MODEL OF OSTEOPOROSIS IN 1X, 30 AND 0/1 POTENCIES"** is a bonafied work carried out by me under the direct supervision and guidance of **Dr. WINSTON VARGHEESE, M.D., (Hom.), PROFESSOR, DEPARTMENT OF MATERIA MEDICA** in partial fulfillment of the Regulations for the award of degree of **DOCTOR OF MEDICINE (HOMOEOPATHY)** in **MATERIA MEDICA** of **THE TAMIL NADU DR. M.G.R MEDICAL UNIVERSITY, CHENNAI**. This has not been submitted in full or part for the award of any degree or diploma from any University.

Place: Kulasekharam **Dr. ALPHY MATHEW**

Date:

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Dr. ALPHY MATHEW

ABSTRACT

Post menopausal osteoporotic changes were induced to a group of selected 30 female Wistar albino rats through ovariectomy. Group 1 received 0.9% saline and served as control and group 2 received estrogen (2 mg/kg). Groups 3, 4 and 5 received Calcarea Carb in potencies 1X, 30 and 0/1 respectively. After 90 days of treatment, the rats were sacrificed for femoral physical parameters, uterine weight, body weight, biochemical parameter and histopathological parameter. Calcarea Carb is found to be effective in controlling osteoporosis with respect to all the above mentioned parameters in all the 3 potencies particularly in 0/1 potency. The study is statistically significant with a p value <0.05.

Keywords: Osteoporosis, Ovariectomy, Calcarea Carbonica, Osteocalcin, Alkaline phosphatase, Calcium, Phosphorous.

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ABBREVIATIONS USED

SL NO:	ABBREVIATIONS	EXPLANATION
1.	BMD	BONE MINERAL DENSITY
2.	GnRH	GONADOTROPIN RELEASING HORMONE
3.	ALP	ALKALINE PHOSPHATASE
4.	IGF	INSULIN-LIKE GROWTH FACTOR
5.	RANK	RECEPTOR ACTIVATOR OF NUCLEAR FACTOR $_{K}$ B
6.	M-CSF	MACROPHAGE- COLONY STIMULATING FACTOR
7.	TNF ∞	TUMOR NECROSIS FACTOR ALPHA
8.	TGF β	TRANSFORMING GROWTH FACTOR BETA
9.	PTH	PARATHYROID HORMONE
10.	PTHrP	PARATHYROID HORMONE RELATED PEPTIDE
11.	IL	INTERLEUKIN
12.	HCl	HYDROCHLORIC ACID

1. INTRODUCTION

Osteoporosis is a disease characterized by low bone mass and micro-architectural deterioration of bone tissue, leading to enhanced bone fragility and an increase in fracture risk [(1)]. It is the most common cause for broken bone among the elderly.

Calcium is an important component of the skeletal tissue which contributes for its strength. In post menopausal age groups, with loss of estrogen, there will also be an alteration in the calcium metabolism resulting in low calcium levels. This can result in reducing the bone mineral density and thus contributing to fractures. Homoeopathy system has a valuable remedy, Calcarea Carb, which is nothing but calcium carbonate, prepared from the middle layer of oyster shell. Calcarea Carb has marked action on the bones [(2)].

From a retrospective study conducted on Calcarea Carb, it was found that Calcarea Carb has a great affinity for musculoskeletal system and also for females between age groups 36-60 years, which mainly includes menopausal women. Menopause can result in osteoporotic changes [(3)].

The present study intent to prove the same on female Wistar albino rats. It is proved that ovariectomy can induce menopause and thereby osteoporosis in rats. Ovariectomised rats are divided into 5 groups consisting of 2 animals each. Out of 5, 3 groups receive Calcarea Carb and remaining 2 groups receive estrogen and vehicle respectively. At the end of the study femoral physical parameters, uterine weight and body weight of rats and biochemical estimation of blood samples are obtained and the results are compared with all the groups.

The potencies selected are 1X, 30 and 0/1. 1X is a material dose. 30 is a medium potency which Hahnemann used to start with in majority of cases. 0/1 is the latest potency invented by Hahnemann.

1.1 BACKGROUND AND JUSTIFICATION OF STUDY

From a retrospective study on Calcarea Carb done at Sarada Krishna Homoeopathic Medical College, it is found that Calcarea Carb has great affinity for musculoskeletal system [3]. Females between 36-60 years have shown more musculoskeletal affection than any other age groups. This age group mainly includes menopausal women. Menopause can bring about changes in body mass index and can result in osteoporotic changes.

In the modern era of evidence based medicine I wish to prove the same experimentally upon ovariectomised albino rats. Ovariectomy can induce menopause and thereby osteoporosis in rats. The study is done using 3 different potencies of Calcarea Carb, i.e, 1X, 30 and 0/1 to see the effectiveness of this remedy in controlling osteoporosis in ovariectomised albino rats.

1.2 SCOPE OF THE STUDY

- There are not much of experimental studies in homoeopathy. This becomes one of the experimental studies in homoeopathy.
- This is an evidence based study.
- This becomes an experimental model for conducting study on other drugs.
- Authenticity of homoeopathic materia medica can be experimentally proved.
- Notion of the current scientific world and laymen about homoeopathic research can be changed.
- Proves that second phase pharmaceutical trials are possible in homoeopathy.
- Unlike clinical trials, experimental study gives an opportunity for publication in journals other than homoeopathic.
- Acceptance by scientific community.
- Inter disciplinary involvement.
- Gives courage to budding homoeopaths to take up animal experiments.

2. AIMS AND OBJECTIVES

- To study the effect of Calcarea Carb, in osteoporotic activity, in ovariectomised rat model of osteoporosis with 3 different potency scales, 1X, 30 and 0/1.
- To compare the effect of Calcarea Carb with estrogen in controlling osteoporosis.
- To study the potency that is more suitable in treating osteoporosis in ovariectomised rat model of osteoporosis.

3. REVIEW OF LITERATURE

3.1 DEFINITION OF OSTEOPOROSIS

Osteoporosis is a skeletal disorder characterized by a loss of bone osteoid that reduces the bone integrity and bone strength leading to an increased risk of fractures [4][5]. Loss of bone tissue is associated with deterioration in skeletal micro-architecture. The WHO operationally defines osteoporosis as a bone density that falls 2.5 standard deviations (SD) below the mean for young healthy adults of the same gender-also referred to as a T-score of -2.5. Post-menopausal women who fall at the lower end of the young normal range (a T-score of >1 SD below the mean) are defined as having low bone density and are also at increased risk of osteoporosis [4].

3.2 EPIDEMIOLOGY

Osteoporosis is the second most common metabolic bone disease in India [6]. Presently it is estimated that more than 200 million people worldwide suffer from osteoporosis. 30% of all post menopausal women, on an average, have osteoporosis in U.S and Europe. At least 40% of these women and 15-30% of men will sustain one or more fragility fracture [7]. Osteoporosis is responsible for more than 1.5 million fractures annually [8]. This includes 3,00,000 hip fracture, approximately 7,00,000 vertebral fracture, 2,50,000 wrist fracture and more than 3,00,000 fracture at other sites. An initial fracture is a major risk factor for a new fracture. In patients who have a previous history of vertebral fracture there is a 2.3 fold increased risk of future hip fracture and a 1.4 fold increase in risk of distal forearm fracture. Hip fracture will have an overall mortality of 15-30%. In that majority of deaths occur within the first 6 months after fracture [7].

Delhi Vertebral Osteoporosis Study found a prevalence of 17.1% of vertebral fractures among 415 female subjects above 50 years enrolled in the study. 159 out of 1,00,000 women above 50 years in Rohtak district, North India were reported to have hip fractures [9]. Another study conducted in Pune reported the prevalence of osteoporosis as 24.6% in men and 42.5% in women above 50 years [10].

3.3 RISK FACTORS

- BMD dependent
    - Female sex
    - Caucasian/Asian
    - Gastrointestinal disease
    - Hypogonadism
    - Immobilization
    - Chronic liver disease
    - Chronic renal disease
    - Low dietary calcium intake
    - Vitamin D deficiency
    - COPD
    - Drugs–heparin,calcineurin inhibitors, anticonvulsants,thiazolinediones, Aromatase inhibitors, anti-androgens, GnRH analogues, proton pump inhibitors.
    - Endocrine diseases- Cushing's syndrome, Hyperthyroidism, Hyperparathyroidism.
    - Other diseases- diabetes mellitus, mastocytosis, multiple myeloma, osteogenesis imperfecta.

- BMD independent
    - Increasing age
    - Previous fragility fracture
    - Family history of hip fracture
    - Low body mass index
    - Smoking
    - Alcohol abuse
    - Glucocorticoid therapy
    - High bone turn over

- Increased risk of falling
- Rheumatoid arthritis [1].

3.4 PATHOLOGY

Bone, a dynamic tissue, is constantly remodeled throughout life. Strength and density of bone is provided by the peculiar arrangement of compact and cancellous bone which aids for both mobility and protection. Bone serves as a reservoir for calcium, phosphorous, magnesium, sodium and other ions required for homeostatic functions. Skeleton is highly vascular and receives approximately 10% of the cardiac output.

Extracellular component of bone comprises of a solid mineral phase and organic matrix. Calcium and phosphate in the form of poorly crystalline hydroxyapatite forms the mineral phase. The organic matrix is made up of type 1 collagen (90-95%) and a heterogeneous non collagenous portion which is formed by serum proteins including albumin. The mineral phase of bone is deposited in the spaces between the collagen fibrils.

Two distinct cell types are required for remodeling of bone. These are:- osteoblasts which produce the bone matrix and osteoclasts which reabsorb the bone matrix. Osteoblasts, derived from the cells of mesenchymal origin, are found on the surface of newly forming bone in its active form. The matrix secreted by osteoblasts is mineralized to form osteocyte, which acts as mechanosensors by communicating signals to surface osteoblasts and their progenitors through the canalicular network. Thus osteocytes serve as master regulators for bone formation and resorption. Osteoblast-derived ALP contributes for bone mineralization by hydrolyzing inhibitors of mineralization. Thus osteoblast activity can be assessed by measuring serum bone – specific ALP.

Multi nucleated osteoclasts are formed by fusion of cells derived from the common precursors of macrophages and osteoclasts. Osteoclast activity can be

assessed by measurement of products of collagen degradation. A balance between the functions of osteoblasts and osteoclasts accounts for normal skeletal development.

Several circulating hormones including estrogens, androgens, vitamin D, and parathyroid hormone (PTH), as well as locally produced growth factors such as IGF I and II, transforming growth factor (TGF) β, parathyroid hormone related peptide (PTHrP), ILs, prostaglandins, and members of the tumor necrosis factor (TNF) super family regulate bone remodeling. These regulate the rate at which new remodeling sites are activated, a process that results initially in bone resorption by osteoclasts, followed by a period of repair during which new bone tissue is synthesized by osteoblasts.

Several factors, such as M-CSF, RANK ligand, osteoprotegerin, produced by osteoblasts or marrow stromal cells allow osteoblasts to control osteoclast development and activity.

M-CSF (Macrophage- Colony Stimulating Factor) plays a key role in forming active osteoclasts through processes that leads to the fusion of osteoclast progenitor cells.

RANK Ligand, a member of TNF family, is expressed on the surface of osteoblast progenitors and stromal fibroblast. RANK Ligand binds to RANK receptor on osteoclast progenitors, stimulating osteoclast differentiation and activation, in a process involving cell-cell interactions [(4)].

Osteoprotegerin, a soluble decoy receptor, secreted by stromal osteoblast lineage [(4)(11)] is capable of binding to RANK Ligand and inhibit osteoclast differentiation. Thus osteoprotegerin neutralizes RANKL [(11)].

Several growth factors and cytokines regulate osteoclast differentiation and function. These include interleukins 1, 6 and 11, TNF and interferon gamma. Most hormones that influence osteoclast function acts indirectly by acting on the cells of osteoclast lineage to increase the production of M-CSF and RANK [(4)].

Parathyroid hormone [PTH] helps in restoring normal levels of extracellular fluid calcium by its direct action upon kidneys and bone, and by its indirect action upon

intestinal mucosa. PTH reduces renal clearance of calcium thus maintaining its normal extracellular fluid level. It increases the rate of bone resorption thus moving calcium from bones to extracellular fluid. It increases the calcium absorption from intestine through the synthesis of 125 dihydroxycholecaciferol. The most rapid changes occur from the action on kidneys, but the largest effect is from action on bones. In prolonged hypocalcaemia, PTH regulates extracellular calcium amount at the expense of bone substance [12].

TNF-∞ secreted by Tcells plays an important role in M-CSF and RANKL-dependent osteocalcin formation. Apart from this it has got direct effect on osteoclast precursors thus contributing to bone resorption [13].

T-cells have estrogen receptors. Once estrogen binds to its receptor site on T-cell, it suppresses the production of TNF∞ by T-cells, thereby preventing the osteoclast bone resorption and bone loss. Thus estrogen decreases the production of M-CSF and RANK. Ovariectomy favors T-cell production of TNF∞. Estrogen increases osteoprotegerin. Estrogen up regulates TGF-β, which is an inhibitor of bone resorption. TGF-β acts directly on osteoclast and decreases its activity and increases its apoptosis [11].

Estrogen prevents bone loss through various effects on bone marrow and bone cells, which result in decreased osteoclast formation ,increased osteoclast apoptosis, and decreased capacity of mature osteoclasts to resorb bone [13] .

Calcitonin directly inhibits osteoclast function by binding to its receptor on the basal surface of osteoclast [4].

Osteocalcin is a protein which is secreted only by osteoblasts [4]. Osteocalcin is a calcium dependent biomarker. It has a strong affinity with the bone matrix, which is responsible for bone mineralization [14]. In osteoporotic women there is a deficiency of calcium and phosphorous, thereby decreasing the formation of hydroxtapatite crystals. This makes the free osteocalcin to circulate in blood. So there is an increase in

osteocalcin concentration in osteoporotic post menopausal women [15]. Osteocalcin is a promising bone formation biomarker to study post menopausal osteoporosis [14].

3.5 CLINICAL FEATURES

Patients with osteoporosis are asymptomatic until a fracture occurs. Osteoporotic spinal fracture may present with acute back pain or gradual onset of height loss and kyphosis with chronic pain. The pain of acute vertebral fracture can occasionally radiate to the anterior chest or abdominal wall and be mistaken for a myocardial infarction or intra-abdominal pathology, but worsening of pain by movement and local tenderness both suggest vertebral fracture [16]. Colles' fracture typically follows a fall on an outstretched arm [1]. Peripheral osteoporotic fractures present with local pain, tenderness and deformity, often after an episode of minimal trauma [16].

There are two types of bone losses – type I and type II. Type I is seen mainly in women in the age group 50-70 years due to accelerated bone loss. The fracture sites are mainly in the vertebrae, distal part of the radius and intracapsular part of hip. Type II is seen above the age of 70 years in women and above 80 years in men. It presents with multiple wedge fractures of the vertebrae and extracapsular fracture of hip, proximal humerus and tibia [17].

3.6 INVESTIGATIONS

Plain radiographs usually show a fracture and may reveal previously asymptomatic vertebral deformities. Bone density can be estimated by:

1. **Dual Energy X-ray Absorptiometry (DXA)** measures areal bone density(mineral per surface area rather than a true volumetric density), usually of the lumbar spine and proximal femur. It is precise, accurate, uses low doses of radiation and is the gold standard in osteoporosis diagnosis.

2. **Quantitative Ultrasound** of the calcaneum. This does not require ionizing radiation and is cheaper than other methods. It cannot be used for diagnostic purposes but is useful as a screening procedure prior to DXA assessment.

3. Quantitative CT scanning allows true volumetric assessment, and distinction between trabaecular and cortical bone. However, it is more expensive, require higher radiation than other techniques, and to date offers no clinical advantage [1].

3.7 REMEDY

CALCAREA CARB:

SYNONYM

Calcarea Ostrearum [18].

PREPARATION

It is prepared from the middle layer of Oyster shell [19]. Hahnemann thought that he could obtain a perfectly pure specimen of the Carbonate of Lime from this layer. But chemically, this is not a pure Carbonate of Lime, for it must contain some of the animal matter belonging to the oyster and also a trace of Calcarea Phos. That is why Dr.Hering proposed to call it Calcarea Ostrearum instead of Calcarea Carb [20].

SPHERE OF ACTION

NUTRITION

-GLANDS

- Cervical
- Mesenteric

-BONES

-SKIN

BLOOD

CHEST

Right Lung

HEART

CHILDREN [2].

DURATION OF ACTION

60 days [21].

PHYSIOLOGICAL ACTION

The Carbonate and particularly the Phosphate of Lime, is an important constituent of all tissues of the body, both hard and soft. It contributes to more than 50% of the substance of the bone and teeth. It thus gives them their solidity [22].

Calcarea Carb makes changes in the composition of blood by modifying the nutrition of the vegetative system, which finally terminate in certain constitutional diseases [18].

Impaired nutrition is the keynote of its action and the glands, skin and bones are instrumental in the changes wrought [21].

Calcium and phosphate ions precipitate as apatite. Apatite is an insoluble substance that helps in the calcification of cartilage into bone formation. During acidic conditions, calcium ions get immobilized from calcium deposits. This can lead to osteoporosis [19].

Here there is an irregularity in the distribution of lime resulting in cartilaginous nature of one bone and bony outgrowths on another bone [23].

SKELETON AND EXTREMITIES

Weight bearing bones such as vertebral column, knee, ankle, hip and shoulder joints are mainly affected. Bones are weak, brittle and rachitic, easily fractured, deformed, dislocated, delayed ossification, delayed union, osteophytes formation, caries of vertebra etc. Pathological fracture, especially of long bone is common [19].

The remedy is characterised by defective assimilation and imperfect ossification. The bone tissues take long time to develop and even after maturing, they remain fragile. This is why curvature of the bones is such a common feature in Calcarea, and that the Calcarea child takes so long in learning to walk. The bones of the leg give way under the heavy weight of the body; so knock-knees and bow legs are often to be met with in Calcarea babies, Fontanelles and sutures take long to close [24]. Tardy development of bony tissues with lymphatic enlargements. Extremities deformed, crooked [25]. Indolence of Calcarea Carb at the level of skeletal framework is seen in the form of delayed milestones and open fontanelles [26].

Rheumatoid pains, as after exposure to wet. Sharp sticking, as if parts were wrenched or sprained. Cold, damp feet; feel as if damp stockings were worn. Cold knees cramps in calves. Weakness of extremities. Swelling of joints, especially knee [21]. Both knees hot, pale, swollen, very sensitive to touch. Necrosis of tarsus. Painful swelling at carpus and itching when touched [27]. Burning of soles of feet. Sweat of hands. Arthritic nodosities. Soles of feet raw. Feet feel cold and dead at night. Old sprains. Tearing in muscles [21]. Rawness of soles of feet from perspiration; blisters and offensive foot sweat [28].

MODALITIES

CAUSATION

Alcohol, Cold, moist winds, Excessive venery, Self-abuse, Injury to lower spine, Over-lifting, Strain, Losses of fluids, Suppressed sweat, Suppressed eruption, Suppressed menses, Fright [29].

AGGRAVATION

Exertion, mental or physical; ascending; cold in every form; water, washing, moist air, wet weather; during full moon; standing.

AMELIORATION

Dry climate and weather; lying on painful side [21].

4. MATERIALS AND METHODS

4.1 STUDY SETTINGS

30 female Wistar albino rats are taken for the study. The rats are divided into 5 groups consisting of 6 animals each.

4.2 SELECTION OF SAMPLES

Healthy female albino rats of about 90 days old, weighing about 150-220g is taken for the study.

4.3 STUDY DESIGN

This is an experimental study done on ovariectomised albino rats.

4.4 INTERVENTION

Calcarea Carb is administered orally in 3 different potencies 1X, 30 and 0/1 to 3 different groups of female Wistar albino rats respectively. The remedy is given in water dose using distilled water, i.e., 5 drops, 3 times daily. Freshly prepared dose is administered each time.

4.5 BRIEF OF PROCEDURES

- Female Wistar albino rats weighing about 150-220 g in the age group of about 90 days were acclimatized to the experimental room at temperature 23+/- 2ºC, controlled humidity conditions (50-55%) and 12 hr light/dark cycle for a period of 1 week.
- Animals were caged with a maximum of 2 animals each in a polypropylene cage and fed with standard food pellets and water.
- After 1 week of acclimatization the animals are divided randomly into 5 groups, containing 6 animals each.
- Ovariectomy is done for all animals in all the groups.

- The rats are anesthetized with a combination of ketamine HCl(80mg/kg) and xylazine (10mg/kg) and ovaries are removed bilaterally.
- The fur on the rat abdomen is completely removed with depilatory cream.
- The connection between fallopian tube and uterus is cut and the ovaries are exposed.
- The uterine horn is returned into the peritoneal cavity after the removal of ovaries.
- The muscle incision is sutured with absorbable suture and skin wounds are closed bilaterally with non-absorbable suture.
- Prophylactic gentamycin (10mg/kg) is administered for 4 days and povidone-iodine solution is applied locally.
- After surgery, the rats were housed individually in polyurethane cages for a period of 1 week to allow recovery and then re-grouped in their home cages.
- Ovariectomised groups receive treatment for 90 days starting from the 15th day of ovariectomy.
- Group 1→receives vehicle (0.9% saline) and serves as ovariectomised control.
- Group 2→orally treated with estrogen (2mg/kg) daily.
- Group 3→orally treated with Calc-Carb 1X.
- Group 4→orally treated with Calc-Carb 30.
- Group 5→orally treated with Calc-Carb 0/1.
- Body weight of all the rats is measured weekly.
- At the end of 90 days, all the rats are deprived of food for overnight.

- On the next day, rats are anesthetized by ketamine HCl (50mg/kg) and blood samples are withdrawn by retro-orbital plexuses [30].

4.6 OUTCOME ASSESSMENT

Uterine weight, body weight and femoral physical parameters are measured and histopathological assessment and biochemical estimation of blood samples of rats are done at the end of the study.

1. Body weight and Uterine weight

- Body weight is measured at the end of treatment.
- Uterine weight is measured and compared with other groups.

2. Femoral Physical Parameters

- Fresh isolated left femurs are weighed using an electronic scale.
- Length of the femurs was measured using a digital slide calipers.
- The length is measured from the proximal tip of the femur head to the distal tip of the medial condyle.
- Bone volume and density were measured by fluid displacement method.

3. Biochemical Estimation

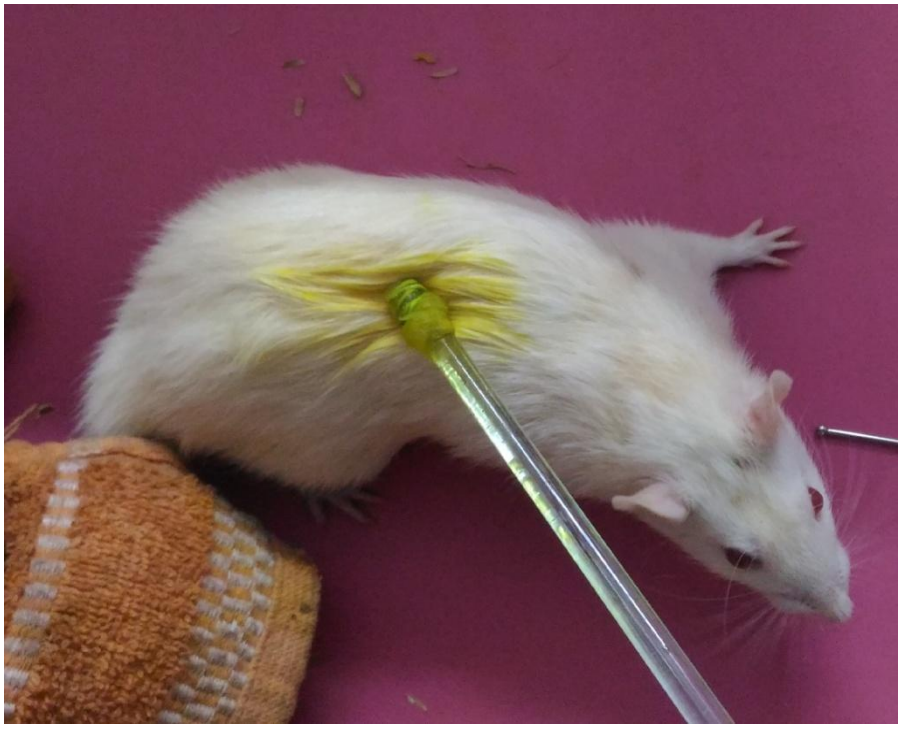
- The levels of serum calcium, phosphorous, alkaline phosphatase and osteocalcin are measured using semi-automatic analyzer with biochemical kits.

4. Histopathology

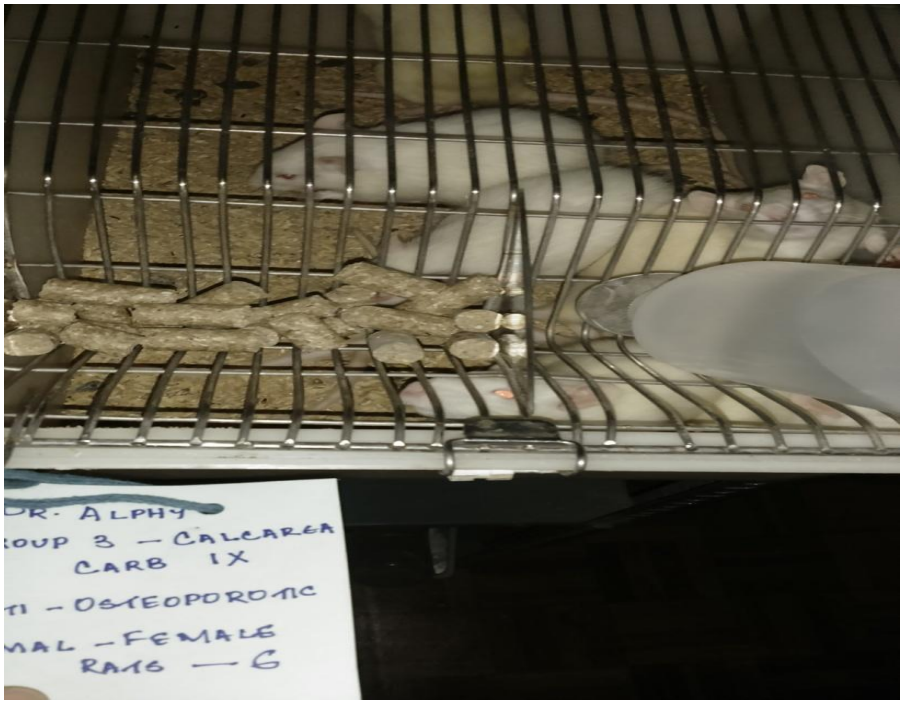
- The right femur was fixed in 10% formalin for 12 hr at 4^0C, decalcified in 5% EDTA for 7 days and embedded in paraffin wax and cut into sagittal plane section of 5µm thickness of the femur.
- The sections were stained with hematoxylin and eosin and examined for histopathological changes under a light microscope [30].

The results are compared with all the groups to see the whether Calcarea Carb and estrogen has similar effect and thereby proving that Calcarea Carb is effective in treating osteoporosis.

4.7 GIVING IDENTIFICATION MARK TO RAT



4.8 RATS IN CAGE



4.9 OVARIECTOMY

4.10 ADMINISTERING MEDICINE

5. OBSERVATION AND RESULTS

5.1 BODY WEIGHT AND UTERINE WEIGHT

TABLE NO.1

5.1.1 BODY WEIGHT

S.NO	GROUPS	INITIAL WEIGHT	FINAL WEIGHT
1.	CONTROL	185.86	190.07
2.	ESTROGEN	173.75	249.90
3.	CALCAREA CARB 1X	176.48	231.76
4.	CALCAREA CARB 30	165.25	215.12
5.	CALCAREA CARB 0/1	166.12	236.82

CHART NO.1

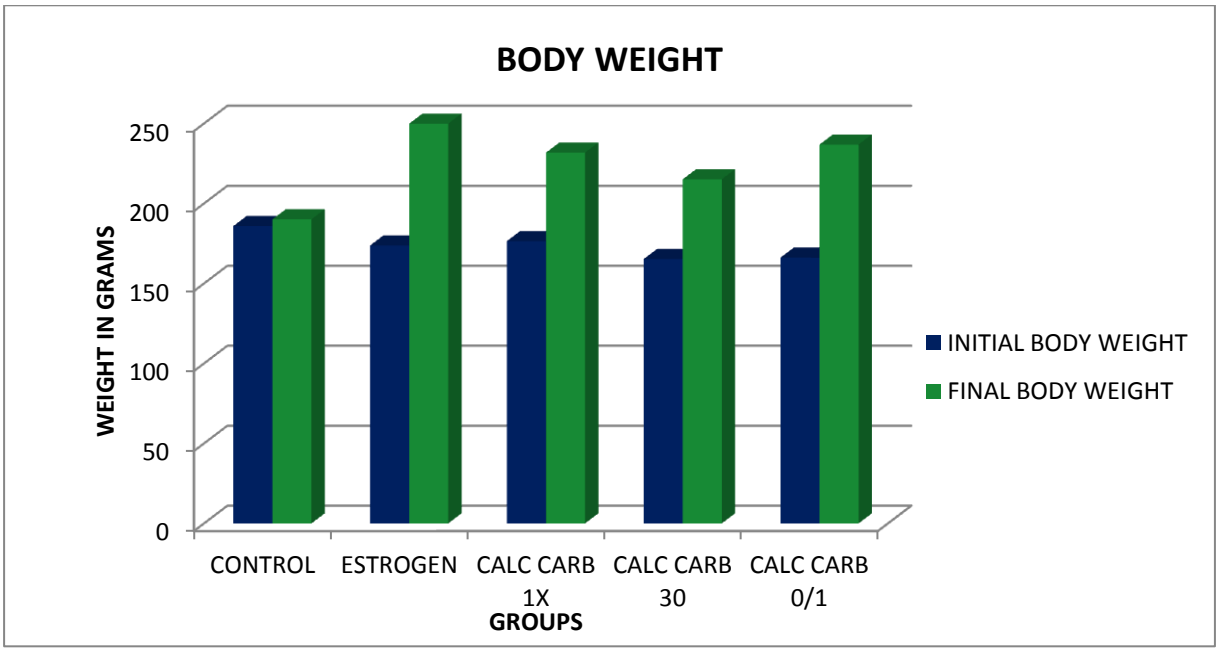
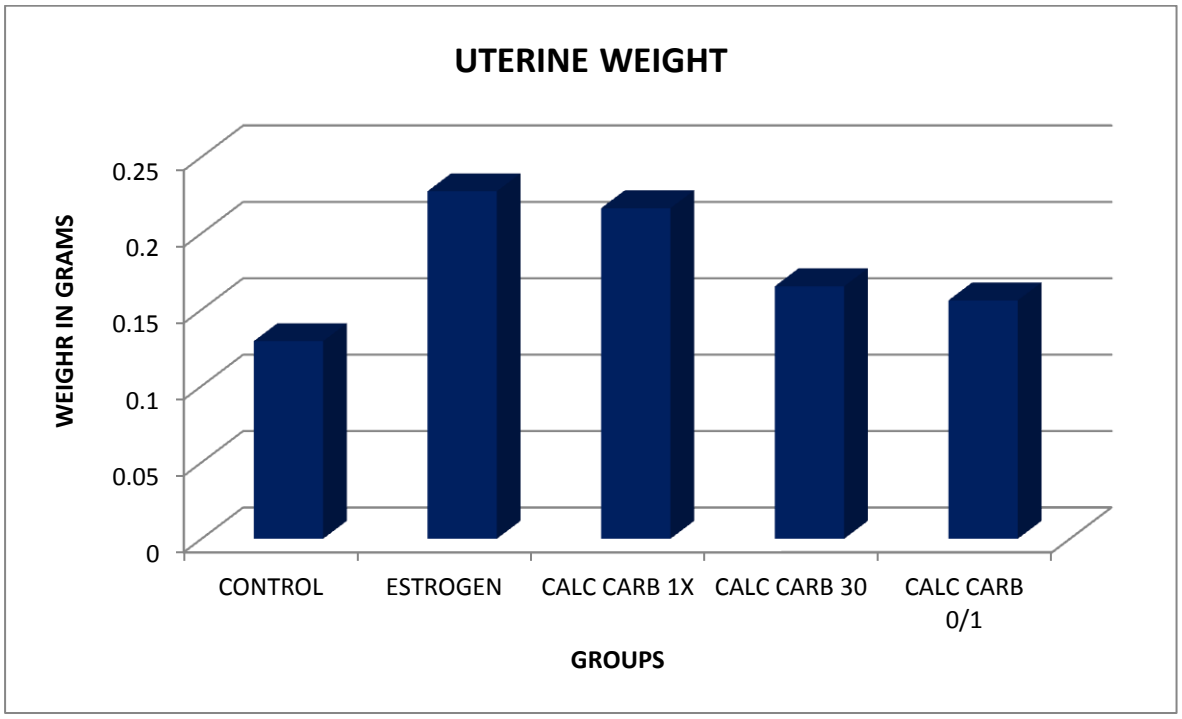


TABLE NO.2

5.1.2 UTERINE WEIGHT

S.NO	GROUPS	UTERINE WEIGHT
1.	CONTROL	0.13
2.	ESTROGEN	0.23
3.	CALCAREA CARB 1X	0.22
4.	CALCAREA CARB 30	0.17
5.	CALCAREA CARB 0/1	0.16

CHART NO.2



5.2 LEFT FEMORAL PHYSICAL PARAMETERS

TABLE NO.3

5.2.1 LEFT FEMUR LENGTH

S.NO	GROUPS	AVERAGE LEFT FEMUR LENGTH
1.	CONTROL	35.47
2.	ESTROGEN	36.16
3.	CALCAREA CARB 1X	36.66
4.	CALCAREA CARB 30	36.15
5.	CALCAREA CARB 0/1	36.31

CHART NO.3

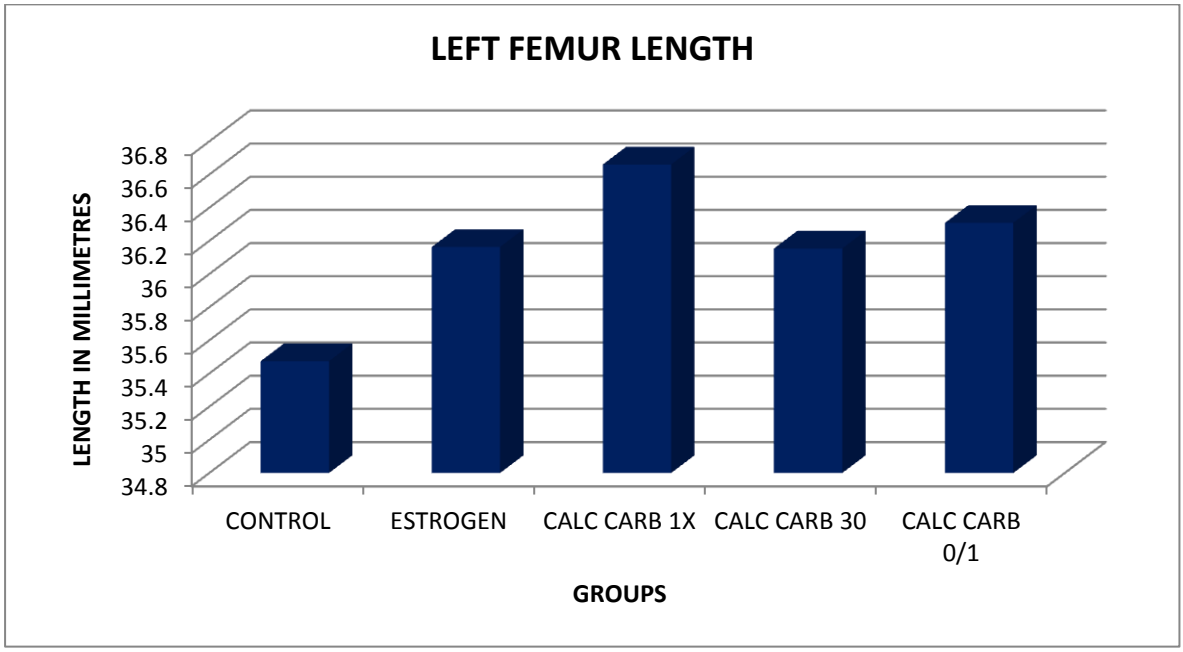


TABLE NO.4

5.2.2 LEFT FEMUR WEIGHT

S.NO	GROUPS	AVERAGE LEFT FEMUR WEIGHT
1.	CONTROL	0.68
2.	ESTROGEN	0.82
3.	CALCAREA CARB 1X	0.75
4.	CALCAREA CARB 30	0.73
5.	CALCAREA CARB 0/1	0.69

CHART NO.4

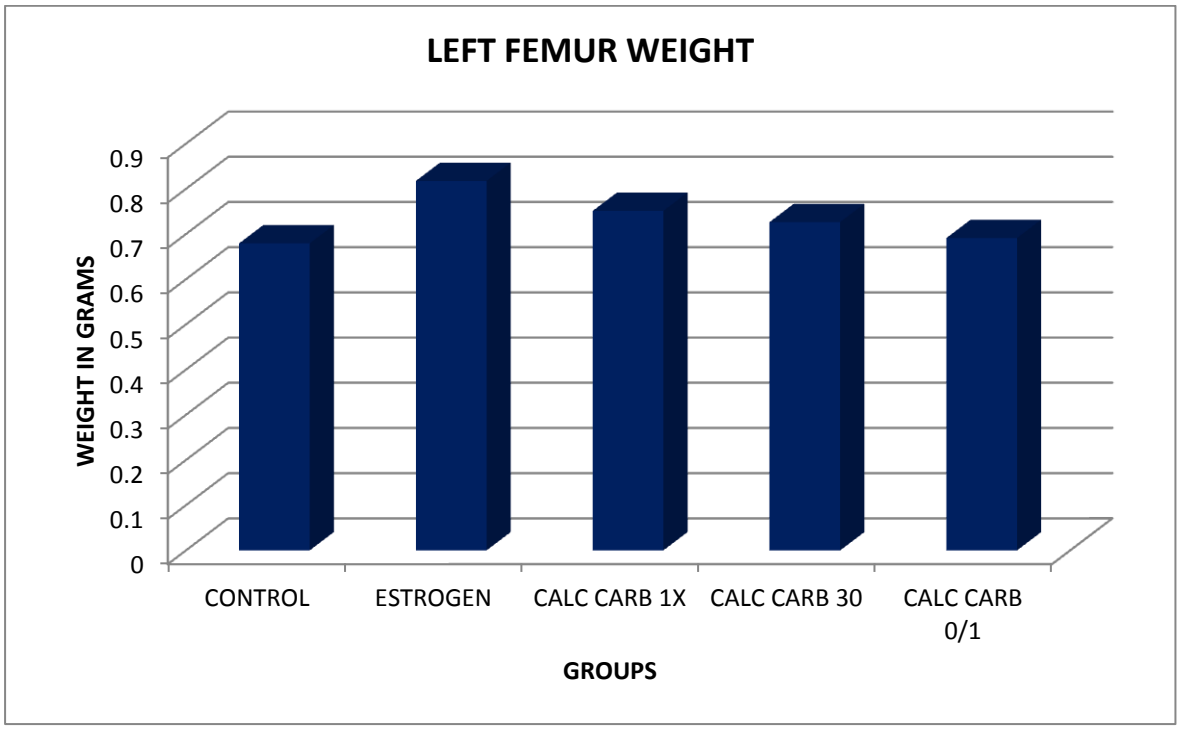


TABLE NO.5

5.2.3 LEFT FEMUR VOLUME

S.NO	GROUPS	AVERAGE LEFT FEMUR VOLUME
1.	CONTROL	0.68
2.	ESTROGEN	0.73
3.	CALCAREA CARB 1X	0.70
4.	CALCAREA CARB 30	0.68
5.	CALCAREA CARB 0/1	0.68

CHART NO.5

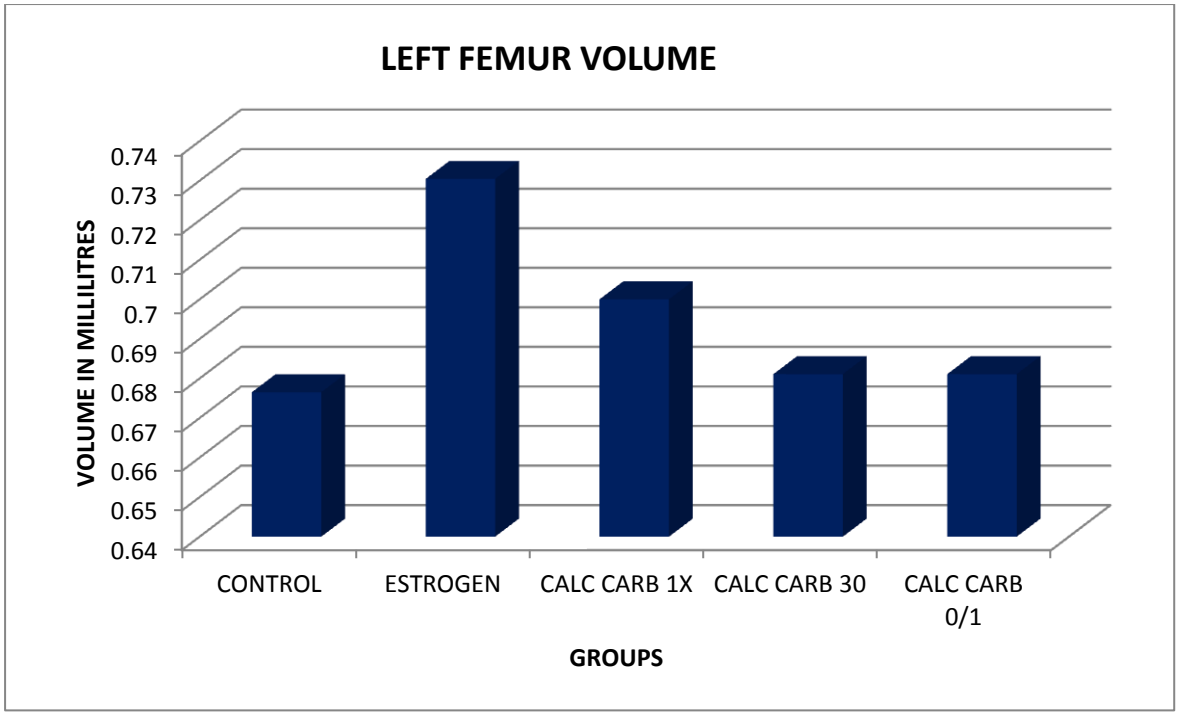
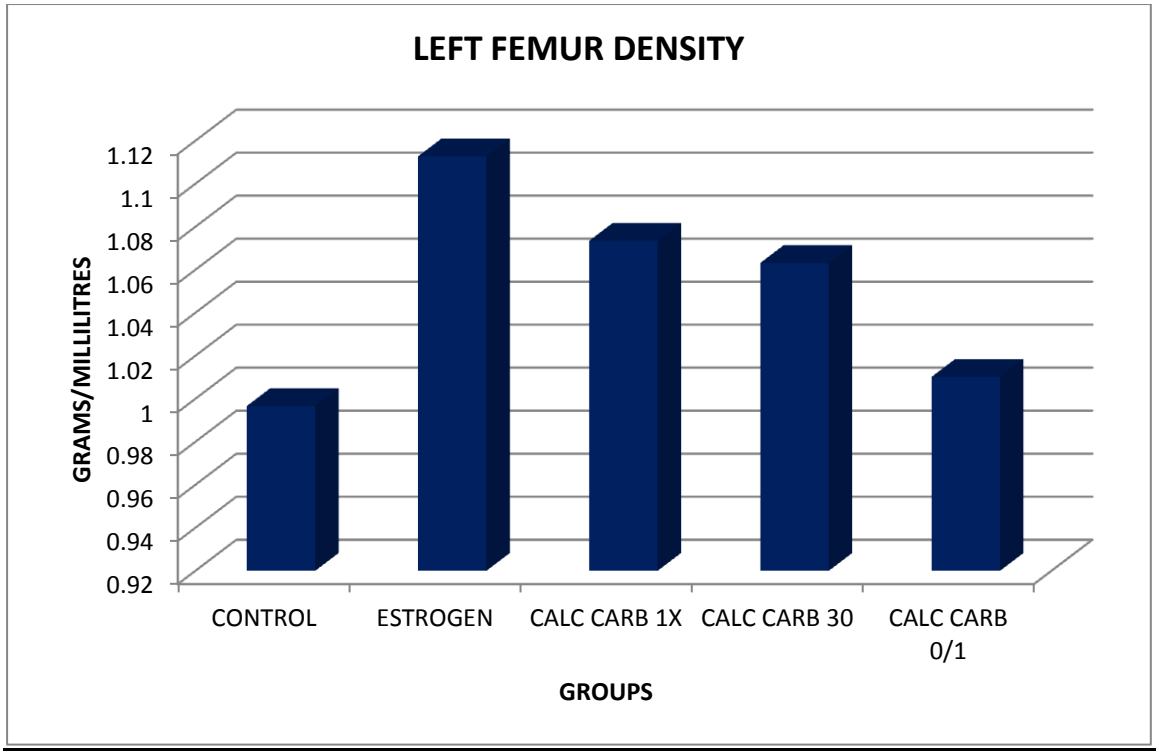


TABLE NO.6

5.2.4 LEFT FEMUR DENSITY

S.NO	GROUPS	AVERAGE LEFT FEMUR DENSITY
1.	CONTROL	0.99
2.	ESTROGEN	1.11
3.	CALCAREA CARB 1X	1.07
4.	CALCAREA CARB 30	1.06
5.	CALCAREA CARB 0/1	1.01

CHART NO.6



5.3 .BIOCHEMICAL ESTIMATION

TABLE NO.7

5.3.1 SERUM CALCIUM LEVEL

S.NO	GROUPS	AVERAGE SERUM CALCIUM LEVEL
1.	CONTROL	6.5
2.	ESTROGEN	7.7
3.	CALCAREA CARB 1X	9.6
4.	CALCAREA CARB 30	8.02
5.	CALCAREA CARB 0/1	7.02

CHART NO.7

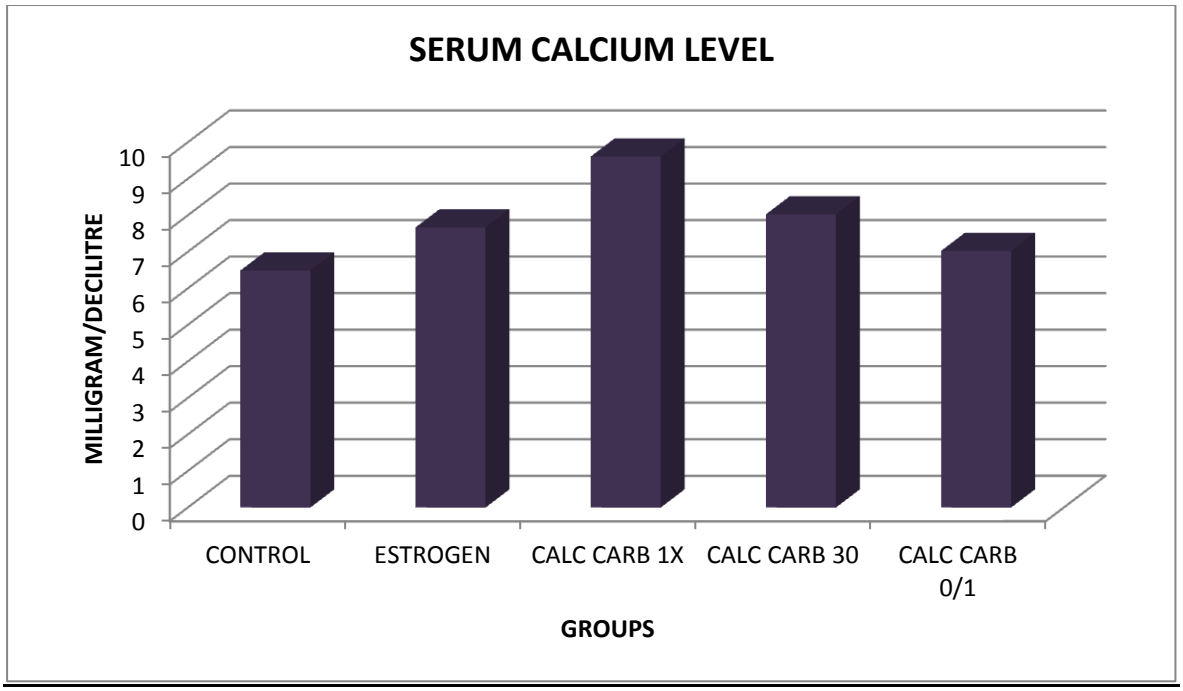


TABLE NO.8

5.3.2 SERUM PHOSPHORUS LEVEL

S.NO	GROUPS	AVERAGE SERUM PHOSPHORUS LEVEL
1.	CONTROL	7.59
2.	ESTROGEN	8.23
3.	CALCAREA CARB 1X	12.42
4.	CALCAREA CARB 30	10.46
5.	CALCAREA CARB 0/1	9.30

CHART NO. 8

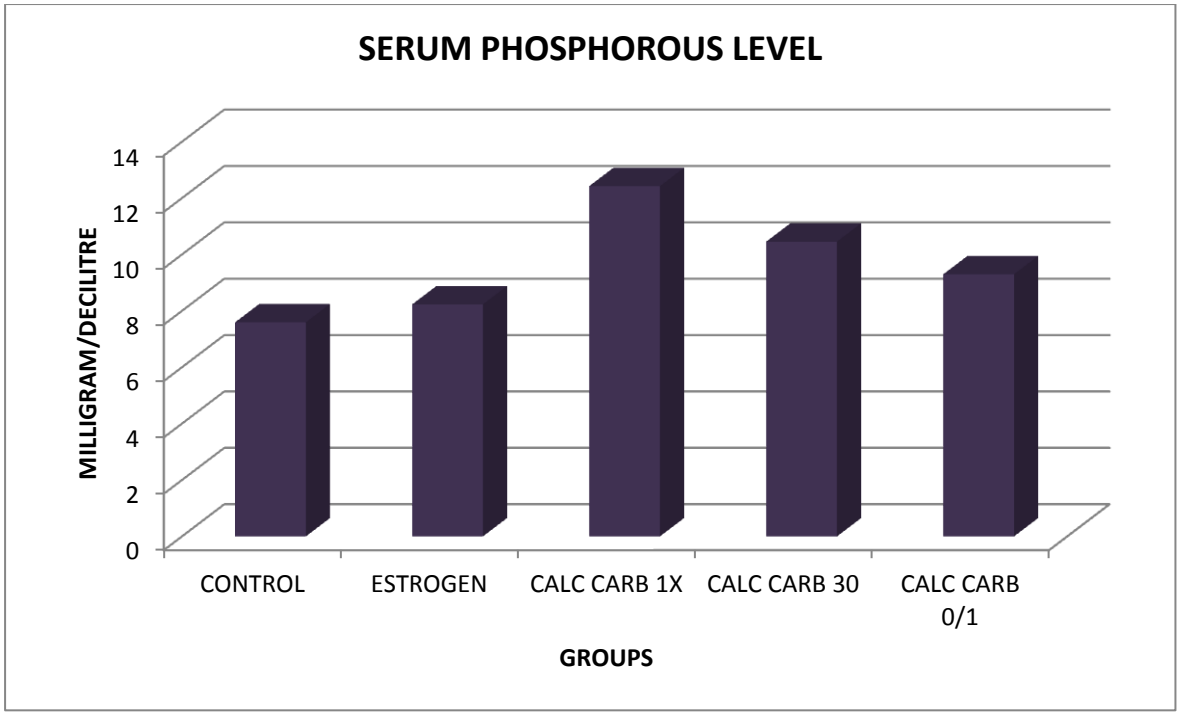


TABLE NO.9

5.3.3 SERUM ALKALINE PHOSPHATASE LEVEL

S.NO	GROUPS	AVERAGE SERUM ALP LEVEL
1.	CONTROL	68.17
2.	ESTROGEN	104.20
3.	CALCAREA CARB 1X	215.80
4.	CALCAREA CARB 30	77.28
5.	CALCAREA CARB 0/1	78.80

CHART NO.9

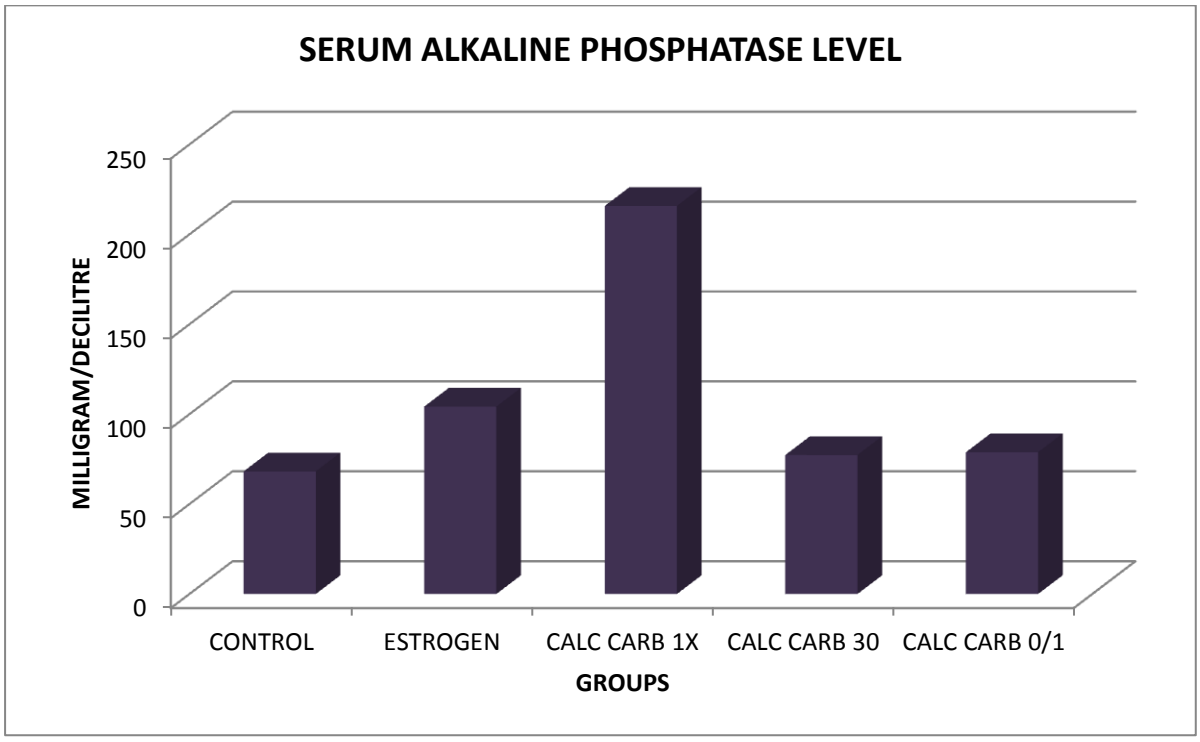
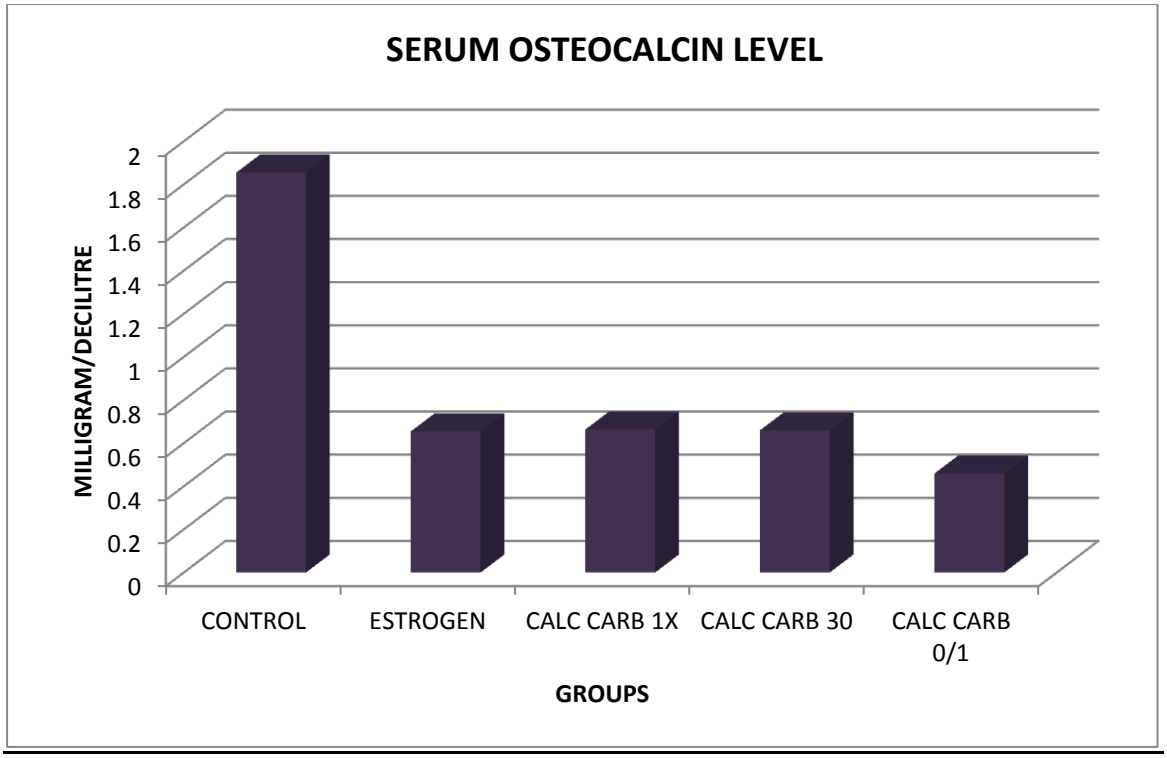


TABLE NO: 10

5.3.4 SERUM OSTEOCALCIN LEVEL

S.NO	GROUPS	AVERAGE SERUM OSTEOCALCIN LEVEL
1.	CONTROL	1.85
2.	ESTROGEN	0.65
3.	CALCAREA CARB 1X	0.66
4.	CALCAREA CARB 30	0.66
5.	CALCAREA CARB 0/1	0.46

CHART NO: 10



5.4 HISTOPATHOLOGICAL ESTIMATION OF RIGHT FEMUR

HISTOLOGICAL CRITERIA FOR OSTEOPOROSIS : [31]

Score	Hip joint cartilage integrity	Structure of trabecular bone	Quantity of trabecular bone
0	Cartilage complete	Normal	90- 100%
1	Cartilage complete	Partially reduced	60-90%
2	Cartilage partially complete	Markedly reduced	30-60%
3	Cartilage absent	Absent	0-30%

Group 1

- Fragments of bony tissue showing endochondral ossification.
- Marrow elements and fat observed.
- Bone edges show a “moth-eaten appearance” suggestive of increased bone resorption.
- Fragments of bone with thin trabeculae and large empty spaces suggestive of increased bone resorption seen.
- Cartilage integrity-0
- Structure of trabaecular bone-1 to 2
- Quantity of trabecular bone-2
- **Total score-4**

Group 2

- Plenty of marrow elements
- Some of the bony fragments appear thinned and show foci of calcification.
- Bony fragments with new osteoid deposition.

- Bone fragments with foci of cartilage and irregular calcification.
- Endochondral ossification with islands of cartilage and foci of calcification.
- Skeletal muscle fragments seen.
- Cartilage integrity-2
- Structure of trabecular bone-0
- Quantity of trabecular bone-1
- **Total score-3**

Group 3

- Endochondral ossification
- Irregular lines of calcification
- Trabeculae appears thinned out
- Cartilage integrity-0
- Structure of trabecular bone-2
- Quantity of trabecular bone-1
- **Total score-3**

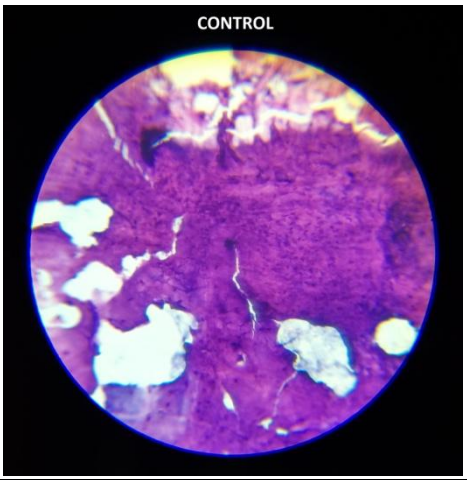
Group 4

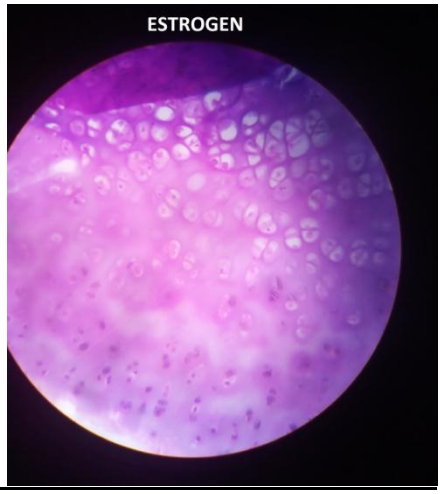
- Endochondral ossification noted
- Osteoid deposition in whirling pattern noted.
- Marrow elements seen.
- Endochondral ossification seen.
- Lamellar bone formation noted
- Cartilage integrity-0 to 1
- Structure of trabecular bone-1
- Quantity of trabecular bone-0
- **Total score-2**

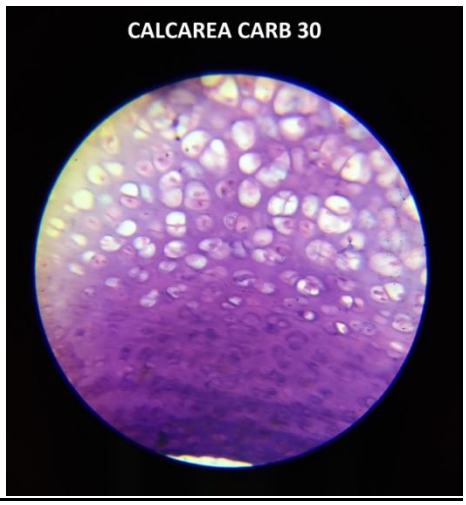
Group 5

- Well formed endochondral ossification and marrow elements.
- Hyaline cartilage
- Woven bone maturing into lamellar bone.
- Well formed muscle and marrow elements.
- Cartilage integrity-0
- Structure of trabecular bone-1
- Quantity of trabecular bone-1
- **Total score-2**

HISTOPATHOLOGICAL OBSERVATIONS:







5.5 OBSERVATION AND RESULT

The average final weight is 190.07 g for control, 249.90 g for estrogen, 231.76 g for Calcarea Carb 1X, 215.12 g for Calcarea Carb 30 and 236.82 g for Calcarea Carb 0/1. After estrogen the highest value for body weight parameter is seen in Calcarea Carb 0/1 which is immediately followed by Calcarea Carb 1X.

The average uterine weight is 0.13 g for control, 0.23 g for estrogen, 0.22 g for Calcarea Carb 1X, 0.17 g for Calcarea Carb 30 and 0.16 g for Calcarea Carb 0/1. After estrogen the highest value for uterine weight was found in Calcarea Carb 1X.

The average left femur length is 35.47 mm for control, 36.16 mm for estrogen, 36.66 mm for Calcarea Carb 1X, 36.15 mm for Calcarea Carb 30 and 36.31 mm for Calcarea Carb 0/1. The highest result was found in Calcarea Carb 1X.

The average left femur weight is 0.68 g for control, 0.82 g for estrogen, 0.75 g for Calcarea Carb 1X, 0.73 g for Calcarea Carb 30 and 0.69 g for Calcarea Carb 0/1. After estrogen, the highest value was found in Calcarea Carb 1X.

The average left femur volume is 0.68 ml for control, 0.73 ml for estrogen, 0.70 ml for Calcarea Carb 1X, 0.68 ml for Calcarea Carb 30 and 0.68 ml for Calcarea Carb 0/1. After estrogen the highest value was found in Calcarea Carb 1X.

The average left femur density is 0.99 g/ml for control, 1.11 g/ml for estrogen, 1.07 g/ml for Calcarea Carb 1X, 1.06 g/ml for Calcarea Carb 30 and 1.01 g/ml for Calcarea Carb 0/1. After estrogen the highest value was found in Calcarea Carb 1 X.

The average serum calcium level is 6.5 mg/dl for control, 7.7 mg/dl for estrogen, 9.6 mg/dl for Calcarea Carb 1X, 8.02 mg/dl for Calcarea Carb 30 and 7.02 mg/dl for Calcarea Carb 0/1. The highest value was found in Calcarea Carb 1X.

The average serum phosphorous level is 7.59 mg/dl for control, 8.23 mg/dl for estrogen, 12.42 mg/dl for Calcarea Carb 1X, 10.46 mg/dl for Calcarea Carb 30 and 9.30 mg/dl for Calcarea Carb 0/1. The highest value was found in Calcarea Carb 1X.

The average serum alkaline phosphatase level is 68.17 mg/dl for control, 104.20 mg/dl for estrogen, 215.80 mg/dl for Calcarea Carb 1X, 77.28 mg/dl for Calcarea Carb 30 and 78.80 mg/dl for Calcarea Carb 0/1. The highest value was found in Calcarea Carb 1X.

The average serum osteocalcin is 1.85 mg/dl for control, 0.65 mg/dl for estrogen, 0.66 mg/dl for Calcarea Carb 1X, 0.66 mg/dl for Calcarea Carb 30 and 0.46 mg/dl for Calcarea Carb 0/1. The lowest value is found in Calcarea Carb 0/1 and highest value is found in control group.

For the histopathological estimation of right femur the total score of control is 4, estrogen is 3, Calcarea Carb 1X is 3, Calcarea Carb 30 is 2 and Calcarea Carb 0/1 is 2. Reduced osteoporosis, better quality cartilage and marrow are found in Calcarea Carb 0/1. . Followed by Calcarea Carb 30, which also scored next lowest in osteoporosis. The treated groups Calcarea Carb 1X and estrogen showed same score with the lamellar thinning most evident in Calcarea Carb 1X. Osteoporosis was most evident in control group.

5.6 STATISTICAL ANALYSIS

5.6.1 ANALYSIS OF VARIANCE

S.NO	ANOVA OF 5 TREATMENT	F VALUE	p VALUE
1.	BODY WEIGHT-FINAL	5.22061	0.003381
2.	UTERINE WEIGHT	2.76282	0.049756
3.	LEFT FEMUR LENGTH	1.71053	0.179175*
4.	LEFT FEMUR WEIGHT	7.78972	0.000321
5.	LEFT FEMUR VOLUME	1.84783	0.151224*
6.	LEFT FEMUR DENSITY	3.25061	0.020566
7.	SERUM CALCIUM	23.85304	<0.00001
8.	SERUM PHOSPHOROUS	38.84629	<0.00001
9.	SERUM ALKALINE PHOSPHATASE	67.16397	<0.00001
10.	SERUM OSTEOCALCIN	21.92886	<0.00001

5.6.2 ‘t’ TEST FOR INITIAL VS FINAL BODY WEIGHT

S.NO	GROUPS	t STAT	p VALUE
1.	CONTROL	0.61	0.5711*
2.	ESTROGEN	13.33	0.00004
3.	CALCAREA CARB 1X	8.49	0.00037
4.	CALCAREA CARB 30	4.18	0.00867
5.	CALCAREA CARB 0/1	6.9	0.00098

‘*’ indicates insignificant values.

5.6.3 CORRELATION BETWEEN STUDY PARAMETERS BETWEEN GROUPS

	FINAL BODY WEIGHT	UTERINE WEIGHT	LEFT FEMUR LENGTH	LEFT FEMUR WEIGHT	LEFT FEMUR VOLUME	LEFT FEMUR DENSITY	CALCIUM	PHOSPH	ALP	OSTEOCA
FINAL BODY WEIGHT	1									
UTERINE WEIGHT	0.2194	1								
LEFT FEMUR LENGTH	0.5771	0.2099	1							
LEFT FEMUR WEIGHT	0.2092	0.4279	-0.0231	1						
LEFT FEMUR VOLUME	0.1617	-0.3280	-0.0703	0.4869	1					
LEFT FEMUR DENSITY	0.1671	0.8064	0.1496	0.7370	-0.1431	1				
CALCIUM	0.2449	0.1030	0.3541	0.3200	0.2938	0.1886	1			
PHOSPH	0.2260	0.0962	0.4368	0.0288	-0.0020	0.1120	0.8642	1		
ALP	0.1970	0.2593	0.3369	0.2639	0.0869	0.2588	0.8279	0.7547	1	
OSTEOCA	-0.5114	-0.2671	-0.3556	-0.4143	-0.3751	-0.3145	-0.4966	-0.4575	-0.2868	1

5.6.4 INTERPRETATION OF STATISTICAL RESULT

From the ANOVA test all parameters are found significant except left femur length and left femur volume. From the‘t’ test for initial v/s final body weight all the groups except control group show a significant p value.

From the correlation between study parameters between groups strong positive correlation is found for final body weight, uterine weight, left femur length, left femur weight, left femur volume, left femur density, calcium, phosphorous, alkaline phosphatase and osteocalcin between all groups. Strong positive correlation is also found between left femur density and uterine weight, left femur density and left femur weight, phosphorous and calcium, ALP and calcium, ALP and phosphorous between all groups. Partial positive correlation exists between left femur length and final body weight between all groups. Negative correlation is found between left femur weight and left femur length, left femur volume and uterine weight, left femur volume and left femur length, left femur density and left femur volume, phosphorous and left femur volume, osteocalcin and uterine weight, osteocalcin and left femur length, osteocalcin and left femur weight, osteocalcin and left femur volume, osteocalcin and left femur density, osteocalcin and calcium, osteocalcin and phosphorous, osteocalcin and ALP between all groups. Remaining parameters are weakly positively correlated between groups.

6.DISCUSSION

Osteoporosis is a metabolic bone disease which represents a very important social and medical problem in developed as well as in developing countries. It is getting more of an epidemic form, as it has a steady increase in the number of cases. After age of 30 years the reduction of bone mass is an unavoidable process, and consequently, alterations in the bone remodeling cycle leads to bone fragility and increased risk of bone fractures**.** With the continuing increase in the number of patients, osteoporosis deserves full attention and appropriate multidisciplinary approach [(32)].

From the study it was found that Calcarea Carb 0/1 ranks highest in average final weight after estrogen. This was followed immediately by Calcarea Carb 1X and Calcarea Carb 30. This indicates that Calcarea Carb helps in maintaining the body weight after menopause and growth parameter was undisturbed by Calcarea Carb.

Calcarea Carb 1X ranks highest in average uterine weight among the Homoeopathic potencies. After menopause, with loss of estrogen, uterine atrophy is a common feature [(33)]. Here Calcarea Carb 1X maintained uterine weight almost equal to that of estrogen, thereby protecting reproductive organs against menopausal changes. This was immediately followed by Calcarea Carb 30 and Calcarea Carb 0/1. This means that Calcarea Carb helped in preventing post menopausal uterine atrophy.

Calcarea Carb 1X ranks highest among homoeopathic potencies in average left femur length, average left femur weight, average left femur volume and average left femur density. Reduction in bone density, bone weight and resultant bone fragility and fractures are the common phenomenon of osteoporosis [(1)(16)]. With estrogenic loss, there is no inhibition to TNF∞; TGFβ and osteoprotegerin production is reduced contributing to increased osteoclastic activity [(11)]. Here estrogen supplementation compensated the loss. Among homoeopathic potencies Calcarea Carb 1X showed beneficial result comparable to estrogen. Calcarea Carb 30 and Calcarea Carb 0/1 also showed beneficial effects after Calcarea Carb 1X. This proves experimentally the authenticity of Homoeopathic materia medica which described the action of Calcarea Carb on bones years back.

Calcarea Carb 1X shows the highest result among all the other groups in serum calcium, phosphorous and alkaline phosphatase levels. Calcarea Carb 30 and Calcarea Carb 0/1 follow it next. This indicates that Calcarea Carb is capable of increasing and maintaining serum levels of calcium, phosphorous and alkaline phosphatase which are very much required for the bone building process [4].

For serum osteocalcin level, Calcarea Carb 0/1 shows the least value and control group shows the highest value among all the other groups. Osteocalcin being a calcium dependent biomarker, will show increased value in case of bone destruction. Its value is less when the bone destruction is minimal [15]. This study result indicates that Calcarea Carb 0/1 has minimum bone destruction whereas control group has maximum destruction compared to all the other groups. This also indicates that Calcarea Carb 0/1has effectively utilized the serum calcium, phosphorous and alkaline phosphatase levels for bone building process and caused very minimal bone destruction.

From histopathological estimation of right femur, it is evident that reduced osteoporosis, better quality of cartilage and marrow are found in Calcarea Carb 0/1. It is immediately followed by Calcarea Carb 30, Calcarea Carb 1X and estrogen. Control group has maximum osteoporotic changes.

Here Calcarea Carb 1X has elevated the physical and biochemical parameters, but has not converted or used serum calcium and phosphorous for deposition and bone formation which is evident from serum levels of osteocalcin and histopathological findings. This indicates that the action of Calcarea Carb 1X is supplementary in nature due to its physiological or material or crude dose. Calcarea Carb 30 is also found to be effective in controlling osteoporosis meeting the needs of bone physically, serologically and histologically. Calcarea Carb 0/1 has not only elevated the physical and biochemical parameters but also has effectively used calcium and phosphorous for deposition and bone formation which is confirmed from the serum osteocalcin and histological observations. This indicates that the dynamic Calcarea Carb 0/1 is the best potency for the bone formation and its action is not supplementary, but curative, not in terms of totally restoring the bony architecture but in terms of effective osteoblastic activity.

7. CONCLUSION

- Calcarea Carb is effective in controlling osteoporosis in ovariectomised albino rat model of osteoporosis in 3 different potency scales i.e, 1X, 30 and 0/1.
- Calcarea Carb 0/1 is effective in controlling osteoporosis by using the elevated biochemical parameters beneficially for osteoblastic activity. It has also taken care of the bone physically and histologically. So Calcarea Carb 0/1 is the best potency for controlling osteoporosis as per the study.
- Calcarea Carb 30 is the next effective potency for controlling osteoporosis, which is evident from the physical, biochemical and histological parameters.
- Calcarea Carb 1X due to its physiological or material nature, has elevated the physical and biochemical parameters to the maximum but has not showed remarkable changes in bony architecture. This confirms that its action is supplementary in nature.
- The action of Calcarea Carb is comparable to that of estrogen in elevating physical and biochemical parameters and acted more than estrogen in histological parameter and serum osteocalcin parameter especially in dynamic doses of Calcarea Carb 30 and Calcarea Carb 0/1.
- p value is less than 0.05 for parameters such as body weight, uterine weight, left femur weight, left femur density and in serum levels of calcium, phosphorous, alkaline phosphatase and osteocalcin, indicating that the test is significant. For left femur volume and left femur length, p value is greater than 0.05, indicating that it is due to chance.

8. SUMMARY

Osteoporosis was induced to a sample size of 30 female Wistar albino rats (divided randomly into 5 groups containing 6 animals each) through ovariectomy after 1 week of acclimatization. After 15 days of post surgical care, these 5 groups received their respective treatment for a period of 90 days after which they were sacrificed for investigations.

It was found that among the homoeopathic potencies Calcarea Carb 0/1 showed highest result in osteoblastic activity by effectively using the calcium, phosphorous and alkaline phosphatase for bone formation. Calcarea Carb 30 was found to be the next effective potency in controlling osteoporosis. It is followed by Calcarea Carb 1X. All the 3 potencies are found to be effective in controlling osteoporosis by taking care of the bone physically, biochemically and histologically. Calcarea Carb has also saved the uterus from post menopausal atrophy and helped in maintaining the body weight. Comparative study with the control group and estrogen group confirmed the same.

The tests are significant, with a p value <0.05, for all the parameters except for left femur length and left femur volume; where it is due to chance.

9. LIMITATIONS

- Individualization is not done in this study.
- Competence of homoeopaths to do an experimental study.
- Acceptance by homoeopaths for an experimental study.
- Challenging the concepts of disease which are dynamic in origin.

9.1 RECOMMENDATIONS

- Studies using animal models of different pathology and different medicine can be done.
- The same study can be done using different medicine.

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